contrary.

Applicant disagrees. Applicants respectfully point out that there is no credible evidence in Nesterenko that the antibodies disclosed therein are the same as those described in the current invention.

Examiner argues that Nesterenko discloses antibodies that bind to a surface antigen proteinase of 24 kD associated with sporozoites of *Crytosporidium parvum* from fecal specimens (abstract and page 77).

Applicants point out to the Examiner that the same Abstract (page 77) also states that "An indirect immunofluorescence assay using these monospecific antibodies revealed that protease occurred on the surface of sporozoites, but **was not** associated with oocyst walls, rhoptries or micronemes." The current specification, page 23, lines 13-34 and page 24, lines 1-9 describes studies performed with the cryptopain inhibitors and results obtained therein. Figure 10 shows the effect of the inhibitors which shows that cryptopain is localized either at the surface, or internally within the sporozoites. These findings contradict statement by Nesterenko who places the protease disclosed in his article only on the surface of sporozoites but not within the oocyst walls or wall organelles, such as rhoptries or micronemes.

Additionally, Nesterenko discloses a **metallo-dependent** cysteine proteinase of **24 kD**, while the current cryptopain has a

sequence of a 401 amino acid protein comprising a **cathepsin L-like** cysteine proteinase of **45 kDa** present in sporozoites and merozoites, and its amino acid and size variants including a deduced mature 226 amino acid protein of MW 25 kDa. In addition to the amino acid sequence, DNA sequence of 1203 nucleotides encoding the 45 kDa cryptopain was also identified.

Clearly, two proteinases are not the same.

Examiner further argues that this surface antigen protease was inhibited by inhibitors of both metalloproteinases and thiol proteinases, but not serine or aspartyl proteinase inhibitors (page 95).

Applicants submit that there are four major classes of proteinases for which the catalytic mechanism has been defined. These proteinases are designated cysteine, aspartic, metallo and serine proteinases (see specification page 15, lines 28-33). The proteinase of Nesterenko is metallo- or thiol-dependent proteinase. Cryptopain of the invention is inhibited by a cathepsin-like cysteine proteinases inhibitors which are not thiol or metallo-dependent, namely inhibitors biotin modified phenyl-alanin-fluoromethyl ketone (BPAFMK), trans-epoxysuccinyl-L-leucylamido-(4-guanidino) butane (E64) and K-III. Results are described in the specification page 23, lines 13-33 and page 24, lines 1-14, and are illustrated in Figures 10A-D.

Examiner will note that cysteine proteinase inhibitors E64 and

K-III are not chemically modified to prevent entry into the cell. The inhibitor BPAFMK is modified to prevent the entry into the cell. Their inhibitory action thus clearly indicates that the proteinase of the invention, which they inhibit at the outside and or the inside of the cell, is not the same proteinase described by Nesterenko.

Another of the Examiner's arguments concerns the sensitivity of the membrane-associated cysteine protease to inhibitors which, according to the Examiner signifies some similarity with the surretn invention in that such sensitivity is similar to that of the metallo-activated cysteine proteinase calpain I and II (page 86).

Applicants submit that this statement is clearly irrelevant in view of the fact that the inhibitors active for the cryptopain are not metallo-dependent. Moreover, the Examiner's argument is clearly supportive of Applicants position in that he quotes page 86 of Nesterenko as stating that the sensitivity of this membrane-associated cysteine protease to inhibitors is similar to that of the metallo-activated cysteine protease calpain I and II. Calpains and cathepsins are distinguished from each other by their cellular locations and by their inhibition profile. For example, cathepsins are inhibited by the peptidyl diazomethane and peptidyl fluoromethylketone inhibitors Z-phe-ala-CHN_2 (diazomethane) and Z-phe-ala-FMK (flucromethylketone; inhibitor BAFMK). Calpains are

not inhibited by these compounds. Cryptopain is inhibited by these coumpounds. Thus the similarity of the sensitivity of Nesterenko's protease to calpains even more distinguishes the current cryptopain from the protease of Nesterenko.

Examiner concludes that the evidence which he cites from Nesterenko seems to suggest that Nasterenko's cysteine protease is the same antigen that comprises SEQ ID Nos: 4-6 of the instant application and, therefore, antibodies to this antigen inherently meet the limitations of the instant claims.

Applicants have shown that the membrane associated cysteine protease of the Nesterenko is not the same as the cryptopain antigen of the invention. Further, Nesterenko does not identify monoclonal antibody, an Examiner's statement in the 103 rejection, with which Applicants agree. Consequently, the current claims cannot be and are not anticipated by Nesterenko. The rejection should be withdrawn and the claims should be allowed. It is so respectfully requested.

Rejections Under 35 USC 103

Claims 19-22 and 25 are rejected under 35 USC 103(a) as being unpatentable over Nesterenko in view of Ramakrishnan et al. ("US 5,817,310, "Ramakrishnan"). The teachings of Nesterenko are set forth above. Nesterenko does not teach monoclonal antibodies. Ramakrishnan does teach the advantage of monoclonal antibodies (column 9, lines 33-46; column 12, lines 1 to column 13, line 65)

which can be produced from immortalized cell lines which would then allow unlimited production of antibodies. It would have been obvious to one of ordinary skill in the art at the time of the invention to make monoclonal antibodies to any pathogen in order that the supply of said antibodies would be steady and constant from an immortalized cell line.

Applicants disagree. Applicants have shown that the protease of Nesterenko is not the same as cryptopain of the invention. Therefore, any combination of the Nesterenko reference with the teaching of Ramakrishnan would not result in the monoclonal antibodies of the invention.

The rejection is overcome and should be withdrawn.

Applicants respectfully request that in view of the submitted arguments Examiner reconsiders his rejection under 35 U.S.C. 102 and 103 and allows the pending claims to issue.

SUMMARY

In summary, Applicants provide arguments overcoming the rejections under 35 U.S.C. 102 and 103. With these rejection being overcome, Applicants believe that the claims are in conditions for immediate allowance. Notice of Allowance is respectfully solicited.

Respectfully submitted,

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Hana Verny (Reg. No. 30,518)
Attorney of Record

PETERS, VERNY, JONES & SCHMITT, LLP
385 Sherman Avenue, Suite 6
Palo Alto, CA 94306
TEL (650) 324-1677 / FAX (650) 324-1678
Atty Dkt: 480.75-2 (HV)

CRYPTOPAIN ANTIBODIES FOR PROPHYLAXIS, TREATMENT, DIAGNOSIS AND DETECTION OF *CRYPTOSPORIDIUM* SPECIES

This application is a based on the provisional application Ser. No. 60/014233 filed on March 27, 1996.

This invention was developed partially with U.S. Government support under National Institutes of Health Grant No U01-AI35123. The U.S. Government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

Field of the Invention

This invention concerns vaccines, antibodies, proteins, DNAs and RNAs for diagnosis, prophylaxis and treatment of *Cryptosporidium* species infections and for detection of *Cryptosporidium* species. In particular, this invention concerns *Cryptosporidium* species antigen comprised of a protein, as well as polyclonal and monoclonal antibodies directed against the antigen, DNAs and RNA encoding the *Cryptosporidium* species antigen and fragments and analogs thereof, and methods for production of recombinant or fusion proteins. This invention also concerns methods for diagnosis, prophylaxis, treatment of *Cryptosporidium* infections and detection of *Cryptosporidium* species.

BACKGROUND AND RELATED DISCLOSURES

The genus *Cryptosporidium* consists of Apicomplexan parasites that invade and develop within epithelial cells of the gastrointestinal, hepatobiliary and respiratory tracts of a wide variety of vertebrates including reptiles, birds and mammals. *Cryptosporidium* was recognized as a cause of animal disease for several decades before the first cases of human cryptosporidiosis were reported in 1976. However, it was not

and recombinantly produced. Cryptopain fusion protein in which the fusion partner is thioredoxin has also been recombinantly produced.

Due to its unique biological activity, cryptopain may be advantageously used for prophylactic, therapeutic, diagnostic and detection purposes.

This invention, therefore, relates to isolated native and recombinantly produced cryptopain; cryptopain amino acid, DNA and RNA sequences; and to vaccines, antibodies, proteins and synthetic proteins, DNAs and RNAs useful for prophylaxis, treatment, diagnosis and detection of infections caused by any *Cryptosporidium* organism or any organism belonging to *Cryptosporidium* species.

More specifically, the invention concerns identification of cryptopain, a *Cryptosporidium* antigen, comprised of a protein or polypeptide, identification of DNA of the *Cryptosporidium* antigen gene within the locus, sequencing DNA encoding the *Cryptosporidium* antigen, expressing portions of the locus encoding the *Cryptosporidium* antigen and using the expressed antigens for preparation of vaccines or for preparation of polyclonal or monoclonal antibodies.

I. Cryptopain - *Cryptosporidium Parvum* Antigen

Cryptopain is cathepsin L-like cysteine proteinase. It is structurally and functionally similar to other cysteine proteinases, represented, for example, by *Carica* papain and *Plasmodium vinckei* cysteine proteinase, and its activity is inhibited by group of cysteine proteinase specific inhibitors.

A. Cysteine Proteinases - Their Function, Structure and Inhibition

There are four major classes of proteinases for which the catalytic mechanism has been defined. These proteinases are designated cysteine, aspartic, metallo and serine proteinases. The major mammalian cysteine proteinases are the lysosomal

recombinant protein of the invention are useful in diagnosing and detecting *Cryptosporidium* as well as for treatment by providing a protection against the *Cryptosporidium* infections.

Anti-*Cryptosporidium* polyclonal antibodies recognizing the cloned polypeptide are preferred over a monoclonal antibody (MAb) because they recognize multiple epitopes on the target polypeptide.

According to the method of the current invention, large amounts of recombinant cryptopain are produced by scale up processes in commercial plants which enables production of a corresponding large quantity of polyclonal antibodies and immunogen for active immunization. The antibodies to recombinant expressed protein can also be produced according to the invention using the standard method available for production of the antibodies to native protein.

Cryptopain comprising epitopes of *Cryptosporidium* that is recognized by intact B and/or T cells is produced in large amounts as described above and in Examples, purified and used to detect or characterize anti-*Cryptosporidium parvum* antibody in the body substances of populations at risk of prior or current cryptosporidial infection. Cryptopain is also used for immunization. Typical intramuscular immunization schedules are as follows.

Cryptopain plus equal volume complete pharmaceutically acceptable adjuvants and excipients is used at the beginning of immunization. Antigen plus equal volume incomplete adjuvant is used at week 2. Antigen plus equal volume incomplete adjuvant at week 4.

In addition, antibodies to such antigens are obtained by immunizing animals, such as rabbits or goats, with the polypeptide plus adjuvant, as described above.

The antibodies of the invention are also used to detect *Cryptosporidium* antigens in body substances, for example,